Inflammation (© 2011)
DOI: 10.1007/s10753-011-9376-7

Interleukin (IL)-10 Gene Polymorphisms Are Associated with Type 2 Diabetes With and Without Nephropathy: A Study of Patients from the Southeast Region of Iran

Mohammad Kazemi Arababadi,[1,2,3] Mohammad Reza Mirzaei,[2,6,7] Seyed Mohammad Ali Sajadi,[4] Gholamhossein Hassanshahi,[2] Behzad Nasiri Ahmadabadi,[3] Vajihe Akbarpour Salehabadi,[2] Reza Derakhshan,[4] and Derek Kennedy[5]

Abstract—The impact of several environmental and genetic factors on diabetes and its complications is well documented. It has also been established that cytokines play a key role in the regulation of immune responses which have been shown to be important in the pathogenesis of diabetes. Studies showed that single-nucleotide polymorphisms within the −592 region of interleukin-10 (IL-10) are associated with the regulation of expression. In this study, we aimed to find polymorphisms of this region that may be associated to type 2 diabetic (T2D) patients with and without nephropathy. In this study, peripheral blood samples were collected from 100 T2D patients without nephropathy, 100 T2D patients with nephropathy, and 100 healthy controls. DNA was extracted, and a polymerase chain reaction-restriction fragment length polymorphism technique was performed to examine the polymorphisms within the −592 region of IL-10 gene. Our results showed a significant difference between the genotypes and alleles of the −592 region of IL-10 in nephropathic and non-nephropathic patients in comparison to the healthy controls. The differences between the two patient groups in relation to genotypes and alleles were not significant. Results of this study suggest that the functional gene polymorphism of IL-10 reported here may play an important role in the pathogenesis of diabetes, but it seems that these polymorphisms do not have an effect on the nephropathic complications of the disease.

KEY WORDS: IL-10; diabetes; nephropathy; polymorphism.

INTRODUCTION

The frequency of diabetes mellitus is increasing globally, and it is expected that this latent disorder will affect 200 million of people by 2010 and 300 million in 2025 [1]. Type 2, sometimes referred to as non-insulin dependent diabetes mellitus or adult-onset diabetes, is the most prevalent type of the diabetes [2]. Current studies showed that several genetic and environmental parameters are associated with type 2 diabetes (T2D) and its complications [3]. It has been suggested that diabetes is an immune-dependent disease in which the pattern of cytokine expression is changed [4]. As an example, in T2D peripheral blood monocytes produce inappropriate levels of inflammatory cytokines which

[1] Department of Microbiology, Hematology and Immunology, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[2] Molecular-Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[3] Infectious and Tropical Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[4] Ali-Ebne Abitaleb Hospital, Rafsanjan University of Medical Science, Rafsanjan, Iran
[5] School of Biomolecular and Physical Science, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan, QLD, Australia
[6] Department of Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[7] To whom correspondence should be addressed at Department of Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. E-mail: mirzaeemr@yahoo.com

may induce the associated pathology of the disease [5]. Subsequently, the cytokine/cytokine–receptor axis has been the subject of several studies to investigate their crucial roles in diabetes and its complications [3], and as a result, the important role of cytokine imbalance in T2D with and without nephropathy has been reported [6, 7]. Increased serum levels of inflammatory cytokines including interleukin (IL)-18 [8], IL-6 [9], IFN-γ [7], IL-17 [7], and TNF-α [9] have been documented in T2D and its nephropathic complications. Furthermore, the association of IL-10 in immunological disorders such as multiple sclerosis [10], systemic lupus erythematosus [11], nephrotic syndrome [12], graft rejection [13], asthma [14], and type 1 [15] diabetes is well established. The key roles of IL-10 as an inhibitory cytokine of autoimmunity and inflammation [16] raise questions concerning the impacts of this cytokine on the pathogenesis of other diseases including T2D and its nephropathic complications. Previous studies showed that the secretion of IL-10 can be affected by polymorphisms in its promoter region [17]. Therefore, this study was aimed to investigate the relation between the polymorphisms of the −592 region of IL-10 in T2D patients with and without nephropathy.

MATERIAL AND METHODS

Subject

Blood samples were collected from 100 type 2 diabetic patients without nephropathy, 100 T2D patients showing nephropathic complications, and 100 healthy controls. All the T2D patients were selected from the Rafsanjan population that had been referred to the Diabetes Clinic of the Ali Ebn-Abitaleb Hospital. The T2D patients were selected based on peripheral blood glucose levels of more than 130 mg/dl, and nephropathy was assessed in diabetic patients based on proteinuria of at least 500 mg/24 h and glomerular filtration rates (GFR) of less than 25 ml/min [18]. GFR were calculated according to creatinine-based equations [19]. The patient and control groups were selected from within the Rafsanjan population with similar medical and demographic characteristics including duration of diabetes, sex, age, and socioeconomic status (Table 1). Assessments of socioeconomic conditions were measured based on the level of education and the monthly income of donors. Classifications for education were: a donor holding a diploma was considered weak, an undergraduate was considered moderate, and a postgraduate was considered high. Monthly income was classified as: under $250, weak; $250–1,000, moderate; and more than $1,000, high. Information regarding lipid levels, proteinuria, estimate of GFR, and drug therapy of patients is also listed in Table 1. Human ethical approval for this study was granted by the Ethical Committee of the Rafsanjan University of Medical Sciences. Consent forms were filled out by both patients and controls prior to blood collection. Previous studies showed that some factors such as infections [20–22], allergic conditions [23], and smoking [24, 25] were considered as bias factors in diabetes and nephropathy; hence, patients with these bias factors were eliminated from the study using a questionnaire.

Assays

Fasting blood sugar, urine albumin level, blood pressure, and clinical presentations were assessed three times during a period of 6 months for each patient and control group.

DNA Extraction

Peripheral blood was collected on EDTA, and genomic DNA was extracted using a commercial kit (Bioneer, Korea) following the manufacturer's recommended procedures. Extracted DNA was aliquoted (for each patient sample) and stored at −20°C for further use.

Detection of Polymorphisms

The IL-10 gene polymorphism (within the gene promoter) was analyzed by PCR-RFLP method. PCR of the promoter region of the IL-10 gene was performed in a volume of 50 μL containing 5 μl of *Taq* DNA polymerase buffer (10×), 1.5 μl of $MgCl_2$ (stock concentration, 1.5 mM), 1 μl of each dNTP [(dATP, dCTP, dGTP, dTTP) at a stock concentration of 10 mM, 2 μl of each primer (stock concentration of 25 ng/μl), 1 μl of prepared DNA, and sterile double distilled water to a final volume of 50 μl. The sequence of the forward primer was 5′-GTAATATCTCTGTGCCTC-3′, and the sequence of reverse primer was 5′-CATTCCAGAATA CAATGG-3′. The amplification was performed using the following program: 1 cycle of 95°C for 5 min (denaturation), 50 s at 53°C for annealing, 72°C for 40 s (elongation) followed by 30 cycles of 95°C for 50 s, 50 s at 53°C for annealing, and 72°C for 40 s using a thermal cycler (C1000, Bio-Rad, USA). During the last 45 s of

t1.1 **Table 1.** Demographic, Socioeconomic Conditions, and Clinical Parameters of T2D Patients with and without Nephropathy and Controls

t1.2 Variant	Healthy control	Type 2 diabetic patients without nephropathy	Nephropathic type 2 diabetic patients
t1.3 Age (years)	40±7	40±9	40±6
t1.4 Sex			
t1.5 Female	60 (60%)	59 (59%)	62 (62%)
t1.6 Male	40 (40%)	41 (41%)	38 (38%)
t1.7 Duration of diabetes (years)		9±3	10±4
t1.8 Socioeconomic status			
t1.9 Weak	22 (22%)	21 (21%)	24 (24%)
t1.10 Medium	47 (47%)	49 (49%)	46 (46%)
t1.11 High	31 (31%)	30 (30%)	30 (30%)
t1.12 Weight (kg)	60±7	50±7	50±9
t1.13 Drug therapy	–	Metformin	Insulin
t1.14 Lipid levels			
t1.15 Triglyceride (mg/dl)	100±4	210±6	350±12
t1.16 Cholesterol (mg/dl)	150±6	170±5.7	290±10
t1.17 HDL (mg/dl)	40±3	35±3	24±2
t1.18 LDL (mg/dl)	100±9	140±6	180±11
t1.19 Glucose level (mg/dl)	95±105	140–190	160–205
t1.20 Proteinuria (mg/dl)	25±1.5	36.6±3	899±50[a]
t1.21 Estimated GFR	120±5	101±5	72±3[b]

t1.22 [a] Significant difference in proteinuria ($p<0.002$, t test, case *vs.* control). Data are shown as mean±SE
t1.23 [b] Significant difference in estimated GFR ($p<0.001$, t test, case *vs.* control). Data are shown as mean±SE

the first stage, 0.3 µl of *T*aq DNA polymerase (5 units) was added to the mixture. The amplified PCR product of promoter region of the IL-10 gene covers the −592 region and has a molecular size of 437 bp. The *R*sa-1 (Fermentase, Finland) restriction site, which was used to detect polymorphisms, is internal to the −592 region which was examined here. *R*sa-1-sensitive fragments were digested into 236 and 201 bp sub-fragments. In the case of heterozygotic patients with the *A/C* alleles, the digest gave rise to three different fragments of 201 and 236 bp and the undigested, 437 bp. In the homozygotic form, a single 437-bp fragment (*C/C* alleles) or the two 201- and 236-bp bands (*A/A* alleles) were observed. The digested products were run on a 2.5% agarose gel (CinnaGen, Iran) and analyzed using a ChemiDoc XRS System (Bio-Rad, USA) after staining with ethidium bromide.

Statistical Analysis

Hardy–Weinberg equilibrium was assessed using the genotype data. Allele and genotype frequencies were calculated in patients and healthy controls by direct gene counting. Statistical analysis of the differences between groups was determined by the χ^2 test using EPI 2000 and SPSS software version 13. A p value of less than 0.05 was considered significant.

RESULTS

Evaluation of polymorphisms within the −592 region of the IL-10 gene by *R*sa-1 restriction digestion showed that the prevalence of the *C/C*, *A/C*, and *A/A* genotypes was 60 (60%), 36 (36%), and 4 (4%) in non-nephropathic T2D patients, respectively (Table 2). Our results also revealed that the frequency of *C/C*, *A/C*, and *A/A* genotypes was 47 (47%), 47 (47%), and 6 (6%) in nephropathic T2D patients, respectively (Table 3). These values for the control group were 22 (22%), 55 (55%), and 23 (23%), respectively (Tables 2 and 3). Statistical analysis showed that the difference between diabetic (nephropathic and non-nephropathic) groups in comparison to the control group in relation to these genotypes was significant ($p=0.001$). Our results also showed that the frequency of *C* allele was 156 (78%), and the *A* allele was 44 (22%) in non-nephropathic T2D patients. The frequency of *C* and *A* alleles was 141 (70.5%) and 59 (29.5%), respectively, in T2D patients with nephropathy (Table 2). Evaluation of the control group showed that the frequency of the *C* allele was 99 (49.5%), and the *A* allele was 101 (50.5%) in this group (Tables 2 and 3). Statistical analysis regarding these alleles showed that the difference between patients (nephropathic and non-nephropathic) in comparison to the control group was also significant ($p=0.001$; Tables 2 and 3).

Table 2. Frequency of Genotypes within the −592 Region of the IL-10 Gene in Non-nephropathic T2D Patients and Controls

Condition	Patients	Control	*p* value
Genotype, *n* (%)			
C/C	60 (60%)	22 (22%)	*p*=0.001
A/C	36 (36%)	55 (55%)	
A/A	4 (4%)	23 (23%)	
Alleles, *n* (%)			
C	156 (78%)	99 (49.5%)	*p*=0.001
A	44 (22%)	101 (50.5%)	

Statistical analysis showed that the frequency of the genotypes and alleles in T2D patients without nephropathy was not different from the T2D patients with nephropathy (p=0.27; Table 4). Our results showed that the difference between the groups in relation to age, sex, and socioeconomic conditions was not significant (Table 1).

DISCUSSION

The crucial role of cytokine networks in orientating immune responses is well documented [26]. Several factors such as infectious agents, hormonal conditions, and cytokine gene polymorphisms regulate expression and secretion of cytokines [27]. The main etiological cause of T2D and its inflammatory complications, such as nephropathy, has yet to be clarified. Some investigators suggested that immune-related factors play important roles in etiology and pathogenesis of T2D and its associated renal complications [28]. As clearly indicated in Table 1, both patients and control groups were demographically matched. Our findings indicated a significant difference between T2D patients with and without nephropathy when compared to healthy controls regarding both genotypes and alleles of the −592 region of the IL-10 gene. Our data revealed that there are no significant differences between the two T2D patient groups; therefore, based on the current results, it can be concluded that the polymorphisms described here are associated with T2D rather than its nephropathic complications in our studied population (southeast Iranian patients). In other words, T2D patients from the Rafsanjanese region show a significant correlation between their disease and the IL-10 −590 polymorphism.

In contradiction to our data presented here, Ezzidi *et al.* showed that the polymorphisms in −592 region of the IL-10 gene are not associated with nephropathic complication in T2D [29]. These finding supported earlier conclusions reported by Scarpelli *et al.*, who had also shown that these polymorphisms are not associated with T2D [30]. On the other hand, Chang *et al.* reported a significant relationship between T2D without nephropathy and healthy controls regarding the −592 region of the IL-10 gene [31]. In addition, Mtiraoui *et al.* reported that the IL-10 promoter polymorphisms of the −1082, −819, and −592 regions are associated with nephropathy complications in T2D [32]. Kolla *et al.* also demonstrated that the polymorphisms within the −1082 region of the IL-10 gene are associated with peripheral neuropathy in South Indian T2D patients [33].

The discrepancies between our results and those of other research groups may be due to differences in the populations that were studied; some of the groups are comprised of different races and genetic backgrounds. It seems that *C/C* genotype is associated with T2D in Rafsanjanese population, and probably, the *C/C* genotype can be considered as a risk factor for the population.

Table 3. Frequency of Genotypes within the −592 Region of the IL-10 Gene in Nephropathic T2D Patients and Controls

Condition	Patients	Control	*p* value
Genotype, *n* (%)			
C/C	47 (47%)	22 (22%)	*p*=0.001
A/C	47 (47%)	55 (55%)	
A/A	6 (6%)	23 (23%)	
Alleles, *n* (%)			
C	141 (70.5%)	99 (49.5%)	*p*=0.001
A	59 (29.5%)	101 (50.5%)	

Table 4. Comparison of the Frequency of Genotypes and Alleles within the −592 Region of the IL-10 Gene Between Nephropathic and Non-nephropathic T2D Patients

Condition	Nephropathic T2D patients	Non-nephropathic T2D patients	p value
Genotype, *n* (%)			
C/C	47 (47%)	60 (60%)	*p*=0.27
A/C	47 (47%)	36 (36%)	
A/A	6 (6%)	4 (4%)	
Alleles, *n* (%)			
C	141 (70.5%)	156 (78%)	*p*=0.27
A	59 (29.5%)	44 (22%)	

JrnlID 10753_ArtID 9376_Proof# 1 - 11/08/2011

Interestingly, our previous study on another anti-inflammatory cytokine (IL-4) showed that T2D patients with nephropathic complication, but not T2D without nephropathy, are significantly associated with IL-4 polymorphisms [34]. Therefore, based on the results of our current and previous studies, it may be concluded that the polymorphisms in IL-10 can affect the onset of T2D, but not nephropathic complication in Rafsanjanese patients, while IL-4 polymorphisms may be correlated to nephropathy in the T2D patients.

In conclusion, nephropathic complications of T2D are very complex; apart from the myriad of cytokines discussed here and reported elsewhere, there is also an association with several environmental and other genetic factors. Significant studies are required to investigate all these parameters and how they influence each other before we can have a comprehensive understanding of the disorder.

ACKNOWLEDGEMENTS

The authors of this article would like to take this opportunity to thank all the diabetic patients and healthy controls who contributed to this research; your dedication is deeply appreciated. We would also like to thank all the staff working at the diabetes clinic in the Ali Ebn-Abitaleb Hospital for their warm cooperation and technical assistance. This project was supported by a grant from the Rafsanjan University of Medical Sciences.

REFERENCES

1. Steyn, N.P., E.V. Lambert, and H. Tabana. 2008. Nutrition interventions for the prevention of type 2 diabetes. *Proc Nutr Soc* 10: 1–16.
2. Zimmet, P., K.G. Alberti, and J. Shaw. 2001. Global and societal implications of the diabetes epidemic. *Nature* 414: 782–787.
3. Nathanson, D., and T. Nystrom. 2008. Hypoglycemic pharmacological treatment of type 2 diabetes: targeting the endothelium. *Mol Cell Endocrinol* 21: 21.
4. Cruz, M., C. Maldonado-Bernal, R. Mondragon-Gonzalez, R. Sanchez-Barrera, N.H. Wacher, G. Carvajal-Sandoval, and J. Kumate. 2008. Glycine treatment decreases proinflammatory cytokines and increases interferon-gamma in patients with type 2 diabetes. *J Endocrinol Invest* 31: 694–699.
5. Giulietti, A., E. van Etten, L. Overbergh, K. Stoffels, R. Bouillon, and C. Mathieu. 2007. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. *Diabetes Res Clin Pract* 77: 47–57.
6. Tsiavou, A., D. Degiannis, E. Hatziagelaki, K. Koniavitou, and S.A. Raptis. 2004. Intracellular IFN-gamma production and IL-12 serum levels in latent autoimmune diabetes of adults (LADA) and in type 2 diabetes. *J Interferon Cytokine Res* 24: 381–387.
7. Arababadi, M.K., R. Nosratabadi, G. Hassanshahi, N. Yaghini, V. Pooladvand, A. Shamsizadeh, H. Hakimi, and R. Derakhshan. 2009. Nephropathic complication of type-2 diabetes is following pattern of autoimmune diseases? *Diabetes Res Clin Pract* 87: 33–37.
8. Skopinski, P., E. Rogala, B. Duda-Krol, A. Lipinska, E. Sommer, J. Chorostowska-Wynimko, J. Szaflik, I. Partyka, and E. Skopinska-Rozewska. 2005. Increased interleukin-18 content and angiogenic activity of sera from diabetic (type 2) patients with background retinopathy. *J Diabetes Complications* 19: 335–338.
9. Pickup, J.C., G.D. Chusney, S.M. Thomas, and D. Burt. 2000. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci* 67: 291–300.
10. Suppiah, V., A. Goris, I. Alloza, S. Heggarty, B. Dubois, H. Carton, A. Antiguedad, M. Mendibe, G. McDonnell, A. Droogan, S. Hawkins, C. Graham, and K. Vandenbroeck. 2005. Polymorphisms in the interleukin-4 and IL-4 receptor genes and multiple sclerosis: a study in Spanish-Basque, Northern Irish and Belgian populations. *Int J Immunogenet* 32: 383–388.
11. Lopez, P., C. Gutierrez, and A. Suarez. 2010. IL-10 and TNFalpha genotypes in SLE. *J Biomed Biotechnol* 2010: 838390.
12. Chen, J., and X. Liu. 2009. The role of interferon gamma in regulation of CD4+ T-cells and its clinical implications. *Cell Immunol* 254: 85–90.
13. Amirzargar, M., M. Yavangi, A. Basiri, S.H. Moghadam, F. Khosravi, G. Solgi, M. Gholiaf, F. Khoshkho, F. Dadaras, M. Mahmmodi, B. Ansaripour, A. Amirzargar, and B. Nikbin. 2007. Genetic association of interleukin-4, interleukin-10, and transforming growth factor-beta gene polymorphism with allograft function in renal transplant patients. *Transplant Proc* 39: 954–957.
14. Wang, X.H., W. Zhao, S.G. Liu, and X.P. Feng. 2009. Correlation of IL-4 and IL-13 gene polymorphisms with asthma and total serum IgE levels. *Zhonghua Jie He He Hu Xi Za Zhi* 32: 161–164.
15. Luczynski, W., A. Stasiak-Barmuta, A. Juchniewicz, N. Wawrusiewicz-Kurylonek, E. Ilendo, J. Kos, A. Kretowski, M. Gorska, L. Chyczewski, and A. Bossowski. 2010. The mRNA expression of pro- and anti-inflammatory cytokines in T regulatory cells in children with type 1 diabetes. *Folia Histochem Cytobiol* 48: 93–100.
16. Sanjabi, S., L.A. Zenewicz, M. Kamanaka, and R.A. Flavell. 2009. Anti-inflammatory and pro-inflammatory roles of TGF-beta, IL-10, and IL-22 in immunity and autoimmunity. *Curr Opin Pharmacol* 9: 447–453.
17. Karjalainen, J., J. Hulkkonen, M.M. Nieminen, H. Huhtala, A. Aromaa, T. Klaukka, and M. Hurme. 2003. Interleukin-10 gene promoter region polymorphism is associated with eosinophil count and circulating immunoglobulin E in adult asthma. *Clin Exp Allergy* 33: 78–83.
18. Momeni, A., S. Shahidi, S. Seirafian, S. Taheri, and S. Kheiri. 2011. Effect of allopurinol in decreasing proteinuria in type 2 diabetic patients. *Iran J Kidney Dis* 4: 128–132.
19. Brown, S. 2010. Re: estimation of glomerular filtration rate with creatinine-based *versus* cystatin C-based equations in kidney transplant recipients. *Iran J Kidney Dis* 4: 169–170.
20. Enriquez, R., A.E. Sirvent, E. Andrada, C. Escolano, J.C. Rodriguez, I. Millan, F. Gutierrez, and F. Amoros. 2010. Cryoglobulinemic glomerulonephritis in chronic hepatitis B infection. *Ren Fail* 32: 518–522.
21. Hrbacek, J., P. Konopasek, V. Eis, E. Hamsikova, R. Tachezy, J. Pokorny, K. El-Balouly, M. Urban, and J. Heracek. 2011. Urologic complications of HIV infection. *Cas Lek Cesk* 149: 115–119.

22. King, G.L. 2008. The role of inflammatory cytokines in diabetes and its complications. *J Periodontol* 79: 1527–1534.
23. Murugan, A.T., and G. Sharma. 2008. Obesity and respiratory diseases. *Chron Respir Dis* 5: 233–242.
24. Fagard, R.H., and P.M. Nilsson. 2009. Smoking and diabetes—the double health hazard! *Prim Care Diabetes* 3: 205–209.
25. Cignarelli, M., O. Lamacchia, S. Di Paolo, and L. Gesualdo. 2008. Cigarette smoking and kidney dysfunction in diabetes mellitus. *J Nephrol* 21: 180–189.
26. Arababadi, M.K., N. Naghavi, G. Hassanshahi, and M. Mahmoodi. 2009. Is CCR5-Delta32 mutation associated with diabetic nephropathy in type 2 diabetes? *Ann Saudi Med* 29: 413.
27. Arababadi, M.K., A.A. Pourfathollah, A. Jafarzadeh, and G. Hassanshahi. 2010. Serum levels of interleukin (IL)-10 and IL-17A in occult HBV infected south-east Iranian patients. *Hepat Mon* 10(1): 31–35.
28. Arababadi, M.K., A.A. Pourfathollah, S. Daneshmandi, G. Hassanshahi, E.R. Zarandi, A. Shamsizadeh, M.A. Rezaei, and S. Eigder. 2009. Evaluation of relation between IL-4 and IFN-g polymorphisms and type 2 diabetes. *Iran J Bas Med Scie* 12(2): 100–104.
29. Ezzidi, I., N. Mtiraoui, M. Kacem, S.G. Mallat, M.B. Mohamed, M. Chaieb, T. Mahjoub, and W.Y. Almawi. 2009. Interleukin-10-592C/A, -819C/T and -1082A/G promoter variants affect the susceptibility to nephropathy in Tunisian type 2 diabetes (T2DM) patients. *Clin Endocrinol (Oxf)* 70: 401–407.
30. Scarpelli, D., M. Cardellini, F. Andreozzi, E. Laratta, M.L. Hribal, M.A. Marini, V. Tassi, R. Lauro, F. Perticone, and G. Sesti. 2006. Variants of the interleukin-10 promoter gene are associated with obesity and insulin resistance but not type 2 diabetes in Caucasian Italian subjects. *Diabetes* 55: 1529–1533.
31. Chang, Y.H., C.N. Huang, C.Y. Wu, and M.Y. Shiau. 2005. Association of interleukin-10 A-592C and T-819C polymorphisms with type 2 diabetes mellitus. *Hum Immunol* 66: 1258–1263.
32. Mtiraoui, N., I. Ezzidi, M. Kacem, M. Ben Hadj Mohamed, M. Chaieb, A.B. Haj Jilani, T. Mahjoub, and W.Y. Almawi. 2009. Predictive value of interleukin-10 promoter genotypes and haplotypes in determining the susceptibility to nephropathy in type 2 diabetes patients. *Diabetes Metab Res Rev* 25: 57–63.
33. Kolla, V.K., G. Madhavi, B. Pulla Reddy, B.M. Srikanth Babu, J. Yashovanthi, V.L. Valluri, J. Ramesh, and J. Akka. 2009. Association of tumor necrosis factor alpha, interferon gamma and interleukin 10 gene polymorphisms with peripheral neuropathy in South Indian patients with type 2 diabetes. *Cytokine* 47: 173–177.
34. Arababadi, M.K. 2010. Interleukin-4 gene polymorphisms in type 2 diabetic patients with nephropathy. *Iran J Kidney Dis* 4: 302–306.